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FINAL REPORT:

Battelle Project No. 48660

July 2005

Studies Supporting the Refinement and Validation of a PBPK Model for 1,4-Dioxane

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ABSTRACT

1,4-Dioxane (dioxane) is a colorless liquid widely used for its solvent properties. Two physiologically based pharmacokinetic models (PBPK) have been developed to describe the disposition of dioxane in rats and humans. One of these models was extended to mice. However, there were notable differences between models in the derivation of partition coefficients and metabolic rate constants even though similar data sets were used in their development. Further, no data are available to validate the model in the mouse. Thus, studies were conducted to determine the partition coefficient values for dioxane in rat, mouse and human blood, and selected rat and mouse tissues. In addition, a kinetic study was conducted to determine blood concentrations of dioxane and its primary metabolite, β-hydroxyethoxyacetic acid (HEAA) following oral administration of dioxane at a low (20 mg/kg body weight), mid (200 mg/kg body weight) or high (2000 mg/kg body weight) dose. At various time intervals ranging from 0.5 to 24 hrs post oral gavage, blood was collected and concentrations of dioxane and HEAA determined. In general, partition coefficient values determined in the present study were found to compare well with previously measured values. For example, the human blood value determined here (1666 $\pm$ 287) confirmed the measured value (1825 $\pm$ 94) reported by Leung and Pastenbach (1990) more closely than the optimized value (3650) derived by Reitz et al. (1990). In the kinetic study, blood dioxane concentrations peaked within 1 hr following administration of a single oral dose and declined thereafter. Similarly, HEAA

blood concentrations peaked between 0.5 to 2 hrs and declined thereafter. Evidence of non-linear metabolism of dioxane was observed as a disproportional increase in the area under the blood concentration-time curve (AUC) with increased administered dioxane dose, along with a decrease in the ratio of HEAA blood AUC and dioxane dose with increased dioxane dose.

INTRODUCTION

1,4-Dioxane (dioxane) is a colorless liquid that has been used as a solvent or as a stabilizer in solvents. Dioxane can be irritating to eyes, skin and the respiratory tract, and can cause lung, liver and kidney damage depending upon the extent and duration of exposure (De Rosa et al., 1996). In chronic drinking water studies, dioxane caused increased incidences in liver tumors in rats and mice, and nasal tumors in mice (De Rosa et al., 1996; Leung and Paustenbach, 1990; Reitz et al., 1990). An extensive database exists describing the toxicity, modes of action and carcinogenic potential of dioxane (reviewed in Stickney et al., 2003). Dioxane is postulated to be primarily metabolized by cytochrome P450s to *p*-dioxane-2-one and β-hydroxyethoxyacetic acid (HEAA), depending on the pH of the analytical methods (Woo et al., 1977; Braun and Young 1977).

Two physiologically based pharmacokinetic (PBPK) models have been developed to describe the disposition of dioxane in rats, mice and humans (Reitz et al., 1990), and in rats and humans (Leung and Paustenbach, 1990). Although both models utilized similar data sets in their development, there were notable differences in the derivation of partition coefficients and metabolic rate constants. Furthermore, there were no data available to validate the Reitz et al. (1990) model in the mouse. Therefore, the purpose of the studies conducted here were to expand upon the existing partition coefficient database by determining blood to air and tissue to air partition coefficients in rat, mouse and human blood and selected rat and mouse tissues, conduct comparative *in vitro* metabolism studies using microsomes from rat, mouse and human liver, and to conduct an *in vivo* toxicokinetic study in mice administered dioxane by oral gavage in a water vehicle.

This report summarizes the results from the partition coefficient and *in vivo* mouse kinetic studies. The *in vitro* metabolism study results will be reported separately.

METHODS AND MATERIALS

Animals. Male Sprague-Dawley rats (300-350 g, approximately 9-11 weeks of age) and male B6C3F1 mice (25-35 g, at least 45 days of age) were obtained from Charles River Breeding Laboratory (Raleigh, NC). Animals were housed in solid-bottom cages with hardwood chips and were acclimated in a humidity- and temperature-controlled room with a 12-h light/dark cycle for at least 5 d prior to use. Certified Purina rodent chow (Ralston Purina Co., St. Louis, MO) and water were provided *ad libitum* throughout the acclimation period. All animal protocols were approved by the Institutional Animal Care and Use Committee at Pacific Northwest National laboratory and studies were performed according to the

"Guide for the Care and Use of Laboratory Animals" (National Research Council, Washington DC, 1996).

*Chemicals***.** 1,4-dioxane was obtained from Aldrich Chemical Co. (St. Louis, MO). HEAA was a gift from Dow Chemical (Midland, MI).

Analytical Methods Development. A method was developed for the simultaneous analysis of dioxane and its major metabolite, HEAA. The additional metabolite, *p*-dioxane-2-one, is converted to HEAA under acidic conditions. A number of methods have been reported in the literature for dioxane in biological or environmental samples using headspace gas chromatography (GC). These methods were utilized for partition coefficient studies. The analysis of *in vivo* samples was conducted using a GC with a mass selective detector (GC/MS) based on a modification of the Young et al. (1976) method. Each analytical method is described below.

Gas chromatographic analysis. Headspace dioxane concentrations were determined using a GC method on an Agilent Model 6890 system (Avondale, PA) equipped with a flame ionization detector (FID). The column was a J&W Scientific (Folsom, CA) DB 624 (30 m x 0.53 mm id x 3.0 µm film thickness). The detector was operated at 275ºC, the inlet at 250ºC, and the final oven temperature was 200ºC. Under these conditions, dioxane had a retention time of approximately 2.1 min. A typical calibration curve is given in Figure 1.

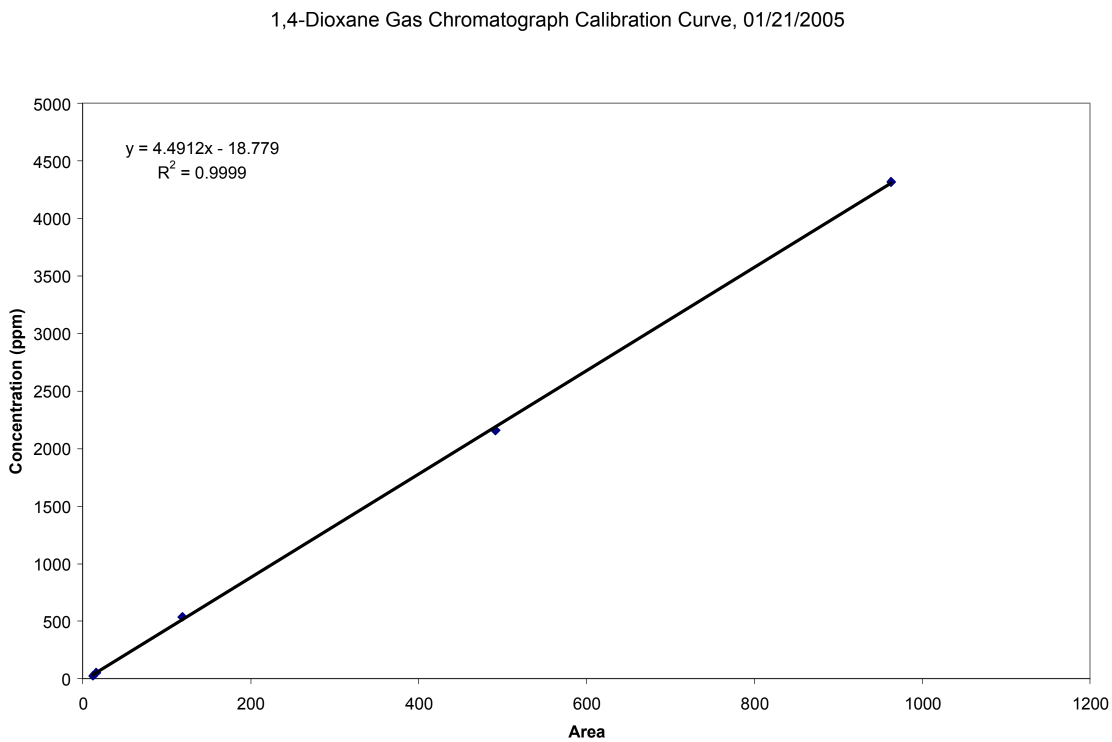

Figure 1: Representative gas chromatography calibration curve for dioxane in air.

Gas chromatography/mass spectrometry analysis. Mouse blood dioxane and HEAA concentrations were determined using a GC/MS detector (model 5973N). The column was a Restek (Bellefonte,PA) Rtx-5MS (3 method on an Agilent Model 6890 GC (Avondale, PA) with a mass selective d 0 m x 0.25 mm id x 0.25 µm df). The GC oven was programmed to ramp from an initial 40ºC to a final temperature of 280ºC at a rate of 18ºC/min. The temperatures of the injection port and MS interface were 200ºC and 280ºC, respectively. For dioxane, 0.1 g blood samples were treated with 0.9 ml of a 1.0 N HCl/internal standard (1,4-dioxane-d-8) solution and extracted using 0.5 ml ethyl acetate. The supernatant was analyzed with quantitation achieved using m/z 88 and 96, for dioxane and 1,4-dioxane-d-8 (internal standard), respectively, both with retention times of approximately 2.4 min). For HEAA, 0.1 g blood samples were treated with 0.9 ml of a 1.0 N HCl/internal standard (glycolic acid) solution and were twice extracted using 0.5 ml tri-n-octylphosphine oxide. The final supernatant was evaporated to dryness under an ultra high purity nitrogen stream and reconstituted with 0.45 ml toluene and 50 µl n-methyl-n-tert-butyldimethylsilyl-trifluoroacetimide (MTBSTFA) and incubated for 1 hr at 60ºC. Quantitation of the supernatant was achieved using m/z 247 for glycolic acid as the internal standard with a retention time of approximately 8 min and m/z 291 for HEAA, with a retention time of approximately 9.9 min. All ions were acquired in scan mode. The limits of reliable quantitation were 0.41 µg/g and 0.39 µg/g for dioxane and HEAA, respectively. Typical dioxane and HEAA calibration curves are given in Figures 2 and 3, respectively.

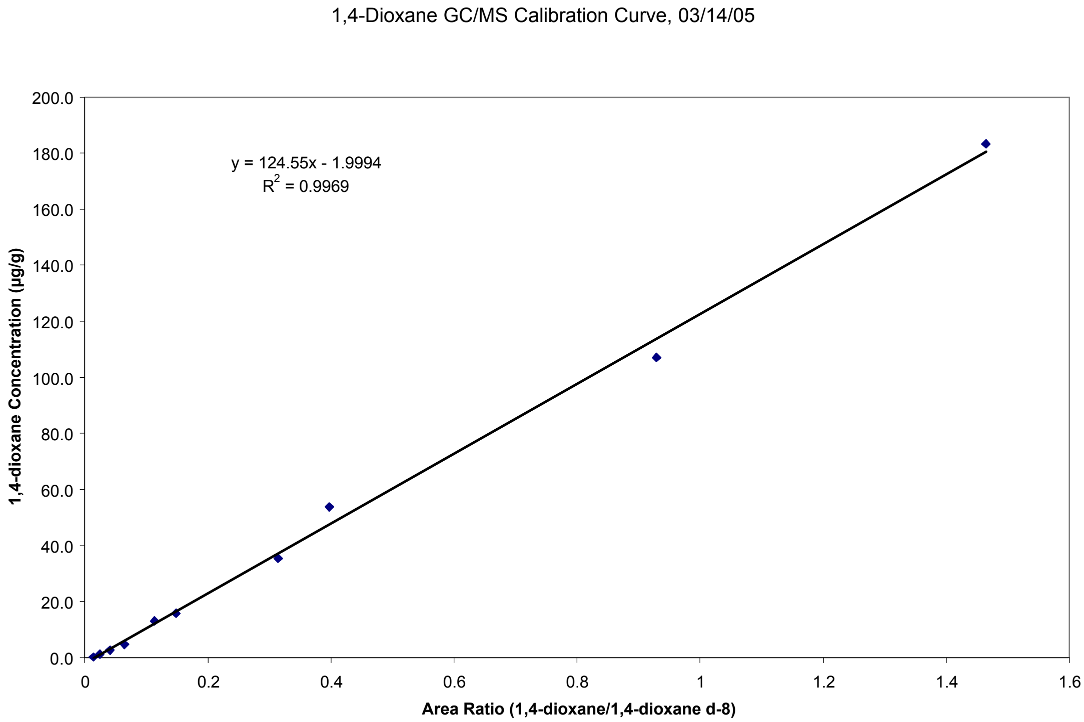

Figure 2: Representative GC/MS calibration curve for dioxane in blood using 1,4-dioxane-d-8 as the internal standard.

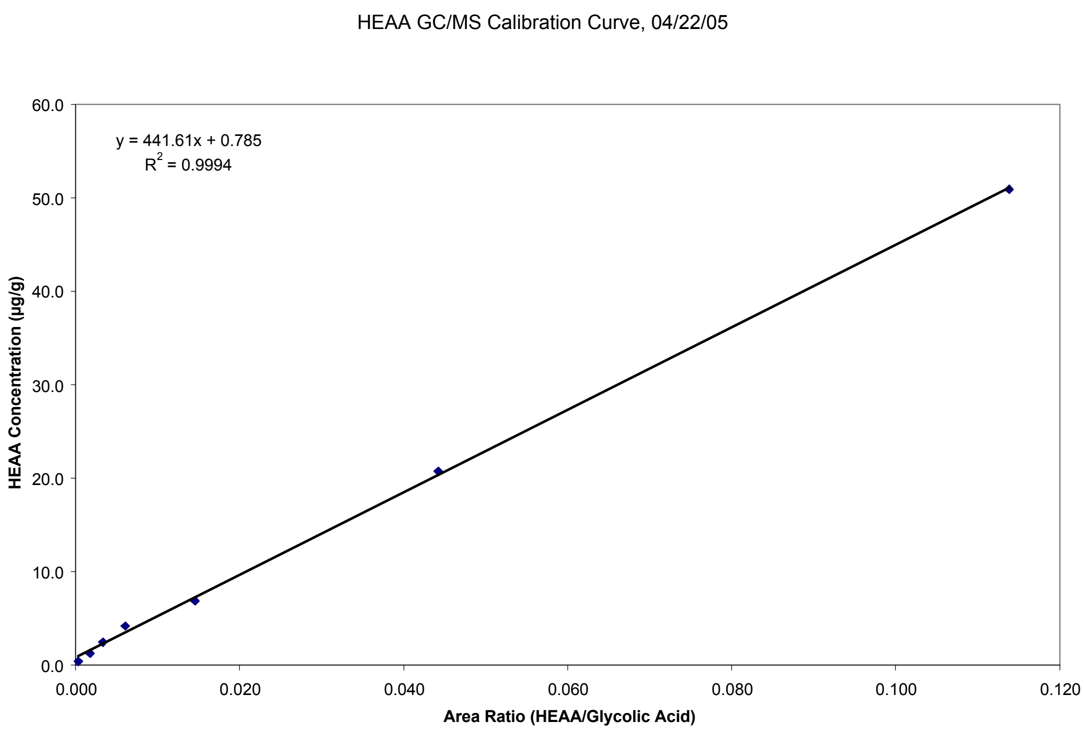

Figure 3: Representative GC/MS calibration curve for HEAA in blood using glycolic acid as the internal standard.

Dioxane Partition Coefficients. Male Sprague-Dawley rats and B6C3F1 mice were euthanized by CO_2 asphyxiation, and heparinized blood was collected by cardiac puncture. Tissues were removed, weighed and stored at -80ºC until use. Heparinized human blood samples from single donors (n=6) were purchased from Golden West Biologicals, Inc. (Temecula, CA). Partition coefficients for blood were determined immediately following collection of the sample using the vial equilibration method described by Gargas et al. (1989). Partition coefficients were determined using 0.5 ml samples of heparinized blood or homogenized tissue (without the addition of saline) weighed into 25-ml glass scintillation vials and sealed air-tight with caps modified by drilling a 4-mm-diameter hole in the center and replacing the liner with a Teflon-coated rubber septum (Supelco Inc., Bellefonte, PA). Dioxane was introduced into the sealed vial as a vapor from a Tedlar gas-sampling bag (SKC-West, Fullerton, CA) containing an air concentration of 21,600 ppm. Vials were incubated at 37ºC with shaking in a vortex evaporator for 3 hr. Preliminary studies indicated no difference in partition coefficient values with 1 hr compared to 3 hr incubations. Headspace dioxane concentrations were determined as described under the Analytical Methods section.

In Vivo Mouse Toxicokinetics. Male B6C3F1 mice (25 to 35 g body weight, at least 45 days of age) were assigned to one of 3 groups (27 mice per group) and administered a single low (20 mg/kg), mid (200 mg/kg) or high (2000 mg/kg) nominal dose of dioxane by oral gavage in a water vehicle. At time intervals of 0, 0.5, 1, 2, 3, 6, 9, 12, and 24 hr post dosing, subgroups of 3 mice/dose group/time point were sacrificed by CO_2 asphyxiation and blood collected by cardiac puncture for analysis of dioxane and HEAA concentrations. Blood samples were stored at -80ºC until analysis; extraction and analysis was conducted according to the GC/MS method described above.

RESULTS AND DISCUSSION

Partition coefficient results. Blood, saline, and tissue to air partition coefficient values for dioxane are provided in Table 1, along with comparative data reported by Reitz et al., (1990) and Leung and Paustenbach (1990). For tissues where comparative data is available, including human blood, the values determined in the current study compare well with those reported by Leung and Paustenbach (1990). Both Leung and Paustenbach (1990) and Reitz et al. (1990) used the same partition coefficient data set for model development; although Reitz et al. (1990) optimized several values to best describe the experimentally observed pharmacokinetics using their model. In particular, Reitz et al. (1990) doubled the experimentally determined human blood to air partition coefficient value given by Leung and Paustenbach (1990) from 1825 ($\pm$ 94) to 3650 to better describe the data using their model. In comparison, the human blood to air partition coefficient value determined here (1666 $\pm$ 287) correlates well with the experimental value reported by Leung and Paustenbach (1990). In addition, in an attempt to improve PBPK model simulation, Reitz et al. (1990) set the kidney partition coefficient equal to the liver value (1557). However, the mouse kidney to air partition coefficient value determined here (560 $\pm$ 175) suggests that the liver might not be a good surrogate for the kidney.

Table 1: Dioxane blood, saline and tissue to air partition coefficient values

	Current Data	Reitz et al., (1990)	Leung and Paustenbach (1990)
Mouse			
Blood	2002 ± 545 (12)[a]	2750	ND[b]
Liver	1143 ± 257 (8)	1557	ND
Fat	879 ± 188 (7)	851	ND
Kidney	560 ± 175 (7)	1557	ND
Muscle	1705 ± 62 (7)	1557	ND
Rat			
Blood	1861 ± 359 (11)	1850	1850 ± 102 (14)
Liver	1862 ± 739 (14)	1557	1557 ± 114 (4)
Fat	ND	851	851 ± 118 (8)
Kidney	ND	1557	ND
Muscle	1348 ± 290 (7)	1557	997 ± 254 (6)
Human			
Blood	1666 ± 287 (36)	3650	1825 ± 94 (14)
Liver	ND	1557	ND
Fat	ND	851	ND
Rapidly Perfused	ND	1557	ND
Slowly Perfused	ND	1557	ND
Saline	2446 ± 269 (8)	2066	ND

[a] Average and standard deviation (when provided). Number in parentheses represents the number (n) of samples.
[b] ND = not determined

In vivo results. Fresh dioxane dosing solutions were prepared on exposure days and concentrations were verified by triplicate analysis of dosing solution aliquots using GC/MS. Each animal received an approximate 0.25 ml volume of the appropriate dosing solution by oral gavage; actual administered volumes were measured as the difference between syringe weight before and after dosing. Animal body weight and dioxane exposure information, along with dioxane and HEAA blood concentration information are provided in the Appendix (Tables A1-A6).

Average blood concentration data for each dose group is summarized in Table 2. For both the high nominal dose (2000 mg/kg body weight) and mid nominal dose (200 mg/kg body weight) groups, peak concentrations occurred within 1 hr following oral dosing and declined thereafter. In contrast, blood dioxane concentrations for the low nominal dose (20 mg/kg body weight) group were at or near background levels throughout the collection time course. However, no efforts were made to confirm whether the dioxane peak at, or near the background concentration was a true dioxane peak or an interfering peak at the same mass and retention time.

Comparisons of blood dioxane and HEAA concentrations for each dose group are given in Figures 4, 5, and 6. As observed with dioxane, HEAA blood concentrations peaked rapidly (0.5 to 2 hrs) and declined thereafter. With the exception of the high dose group, no HEAA was detected in blood samples collected beyond the 9 hr time point (i.e., 12 and 24 hrs). At the high dose group, HEAA was detected in blood samples collected through 24 hrs. Although HEAA was measured in blood samples from all 3 dose groups, the highest levels, as a percent of administered dioxane, were observed with the lowest (20 mg/kg body weight) dose group. A comparison of the blood concentration-time curves (AUCs) for dioxane and HEAA suggests non-linear dioxane metabolism, as illustrated by a disproportional increase in the dioxane AUC with increased administered dose, along with a decrease in the HEAA blood AUC/dioxane dose ratio with increased dioxane dose, as shown in Table 3.

Table 2: Average (± standard deviation) concentrations of dioxane and HEAA in blood of mice following a nominal dose of 20, 200 or 2000 mg/kg.

Sacrifice Time (hr)	Dioxane (µg/g)	HEAA (µg/g)
Low Dose		
0	1.57 ± 0.51	0.49[a]
0.5	1.88 ± 0.67	42.02 ± 13.29
1	1.60 ± 0.28	21.21
2	1.40 ± 0.24	2.70 ± 0.87
3	1.44 ± 0.46	0.85
6	1.11 ± 0.22	(0.43-0.71)[b]
9	1.56 ± 0.76	ND[c]
12	1.37 ± 0.70	ND
24	1.68 ± 0.34	ND
Mid Dose		
0	ND	0.82 ± 0.01
0.5	104.94 ± 12.09	112.93 ± 59.55
1	91.26 ± 14.81	117.28 ± 39.07
2	43.49 ± 21.33	87.33 ± 8.55
3	8.55 ± 0.89	71.88 ± 11.13
6	2.49	3.94 ± 0.87
9	ND	(2.79-13.11)[c]
12	1.90	
24	ND	
High Dose		
0	1.65	1.06
0.5	(1284.10-1358.83)	144.86 ± 24.08
1	1728.80 ± 138.90	(86.37-191.62)
2	1715.89 ± 53.41	(221.82-226.42)
3	1306.04 ± 85.06	202.30 ± 11.50
6	1376.35 ± 248.14	225.25 ± 22.44
9	879.57 ± 73.25	(175.74-212.47)
12	383.54 ± 106.87	162.89 ± 11.75
24	ND	(13.60-31.62)

[a] Single values given when n=1 sample
[b] Range given when n=2 samples
[c] ND – not detectable at a limit of reliable quantitation of 0.41 µg/g for dioxane and 0.39 µg/g for HEAA.

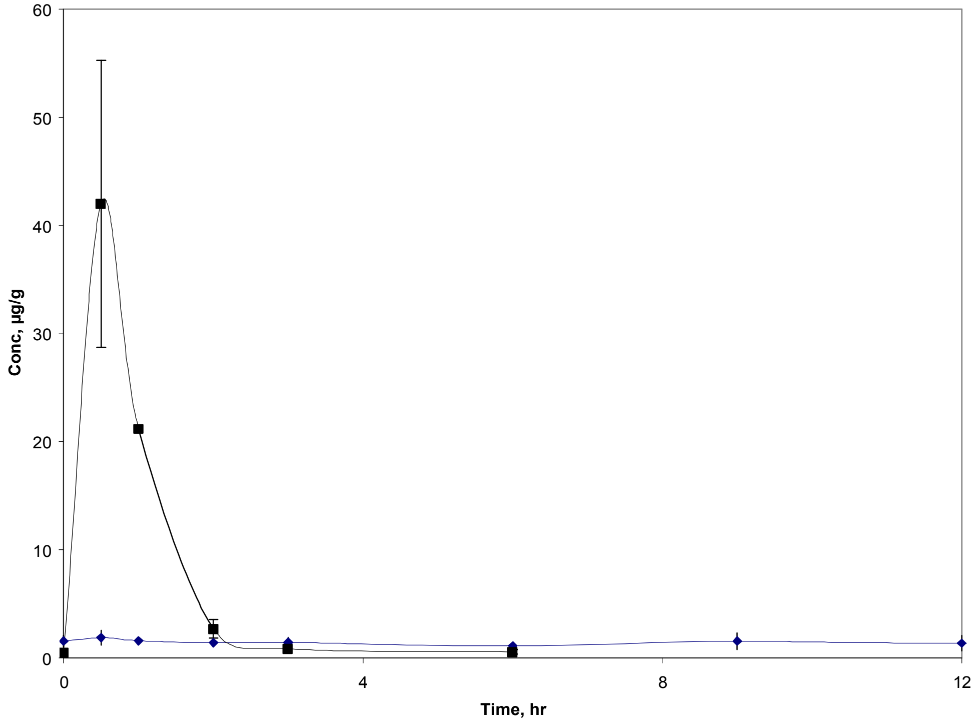

Figure 4: Blood concentrations of dioxane (♦) and HEAA (▪) following a single oral gavage dose of dioxane at the low (20 mg/kg body weight) dose.

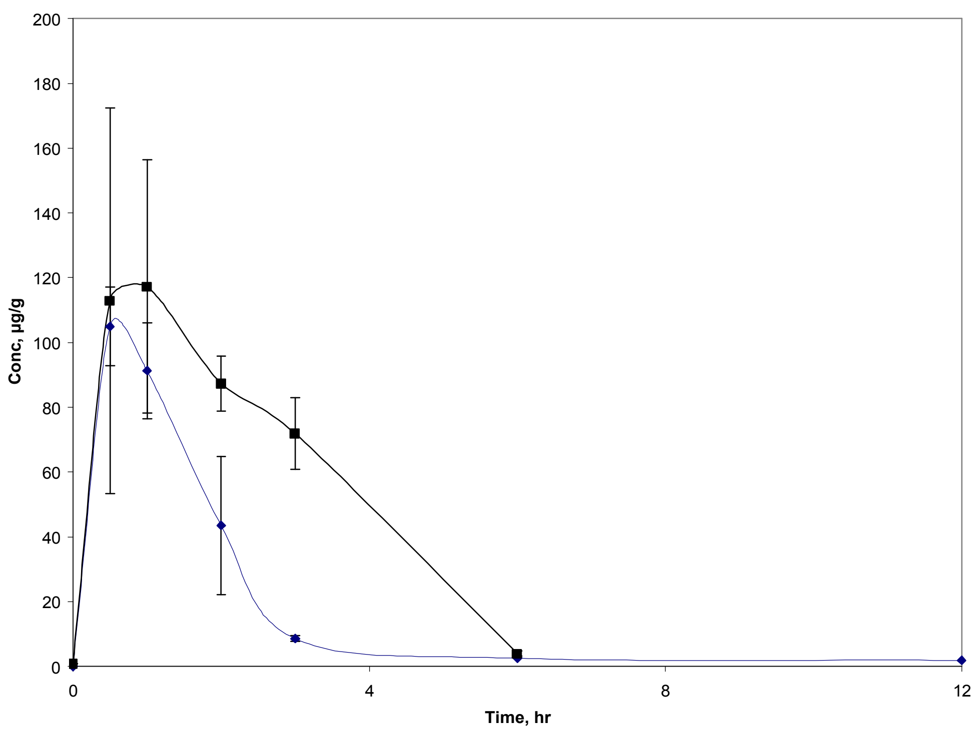

Figure 5: Blood concentrations of dioxane (♦) and HEAA (▪) following a single oral gavage dose of dioxane at the mid (200 mg/kg body weight) dose.

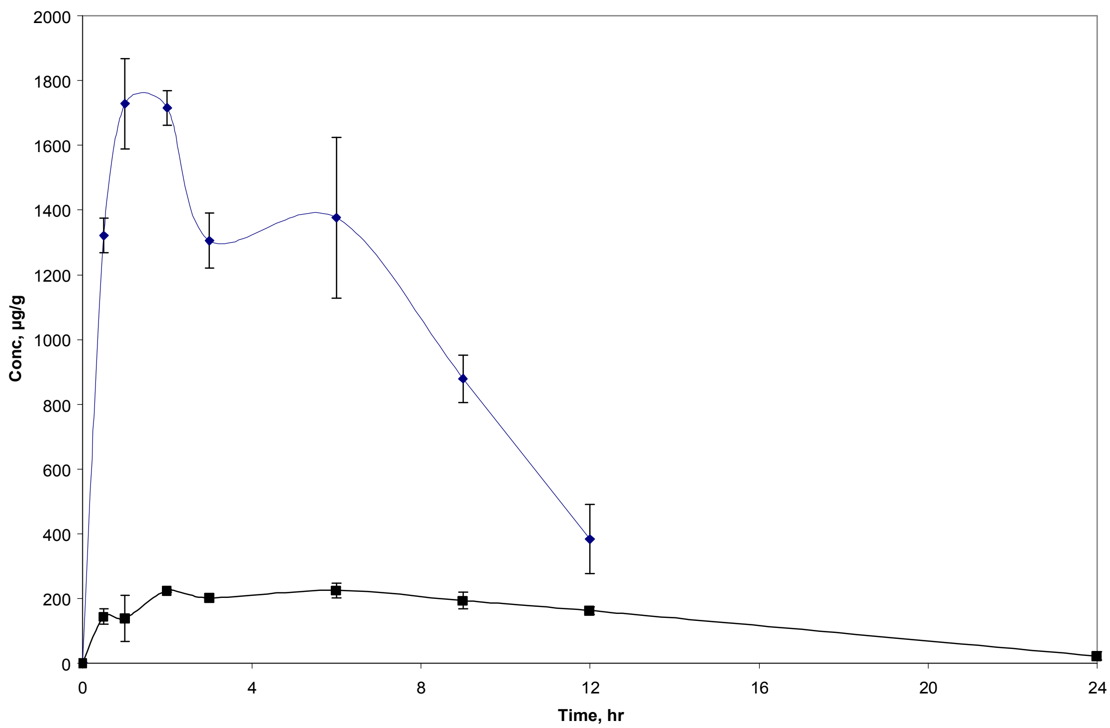

Figure 6: Blood concentrations of dioxane (♦) and HEAA (▪) following a single oral gavage dose of dioxane at the high (2000 mg/kg body weight) dose.

Table 3: Area under the blood concentration-time curve (AUC) for dioxane and HEAA

Actual Dose (mg/kg)	AUC dioxane	AUC HEAA
24.24	16.87	42.29
245.46	198.39	381.63
2230.32	13628.79	1143.53

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APPENDIX

In-Life Kinetic Study Animal Data

Table A-1: Body Weight and Dose Information for Low (20 mg/kg) Dose Group

Sacrifice time (hr)	Body Wt. (g)	Dose Soln. Conc. (mg/ml)	Wt. Administered Dose (g)	Actual Dose (mg/kg)
0	29.3	2.71	N/A[a]	0.00
0	31.1	2.71	N/A	0.00
0	29.4	2.71	N/A	0.00
0.5	25.8	2.72	0.2626	27.68
0.5	29.1	2.72	0.3241	30.29
0.5	30.0	2.72	0.2816	25.53
1	28.2	2.58	0.2574	23.55
1	29.9	2.58	0.2570	22.18
1	29.5	2.58	0.2498	21.85
2	27.6	2.58	0.2380	22.25
2	30.3	2.58	0.3087	26.29
2	28.6	2.58	0.2504	22.59
3	30.0	2.71	0.2307	20.84
3	29.4	2.71	0.2420	22.31
3	26.7	2.71	0.1993	20.23
6	31.6	2.71	0.2515	21.57
6	29.8	2.71	0.2833	25.76
6	31.1	2.71	0.2634	22.95
9	28.2	2.72	0.3096	29.86
9	27.0	2.72	0.2682	27.02
9	28.8	2.72	0.2385	22.53
12	29.9	2.72	0.2104	19.14
12	29.0	2.72	0.2658	24.93
12	29.4	2.72	0.2542	23.52
24	31.1	2.72	0.3136	27.43
24	28.2	2.72	0.2602	25.10
24	28.9	2.72	0.2790	26.26

[a] N/A – not applicable

Table A-2: Body Weight and Dose Information for Mid (200 mg/kg) Dose Group

Sacrifice time (hr)	Body Wt. (g)	Dose Soln. Conc. (mg/ml)	Wt. Administered Dose (g)	Actual Dose (mg/kg)
0	ND[a]	30.16	N/A[b]	0.00
0	31.6	30.16	N/A	0.00
0	29.1	30.16	N/A	0.00
0.5	31.2	26.26	0.2585	217.57
0.5	28.4	26.26	0.2545	235.32
0.5	28.9	26.26	0.2937	266.87
1	27.8	30.16	0.2515	272.85
1	26.1	30.16	0.2679	309.57
1	30.2	30.16	0.2654	265.05
2	27.2	30.16	0.2122	235.29
2	31.3	30.16	0.2661	256.41
2	29.8	30.16	0.2900	293.50
3	34.8	29.16	0.2651	222.14
3	29.9	29.16	0.2446	238.55
3	26.1	29.16	0.2661	297.30
6	29.6	29.16	0.2381	234.56
6	32.5	29.16	0.2567	230.32
6	29.6	29.16	0.2397	236.14
9	26.8	26.26	0.2741	268.58
9	30.8	26.26	0.2070	176.49
9	28.1	26.26	0.2462	230.08
12	25.1	26.26	0.2496	261.14
12	26.3	26.26	ND	N/A
12	32.1	26.26	0.2514	205.66
24	31.5	30.16	0.2271	217.44
24	33.1	30.16	0.2113	192.53
24	28.8	30.16	0.2696	282.33

[a] ND – Not determined. Due to experimental error, body weight was not recorded for the first control animal.
[b] N/A – Not applicable

Table A-3: Body Weight and Dose Information for High (2000 mg/kg) Dose Group

Sacrifice time (hr)	Body Wt. (g)	Dose Soln. Conc. (mg/ml)	Wt. Administered Dose (g)	Actual Dose (mg/kg)
0	29.9	264.65	N/A[a]	0.00
0	28.6	264.65	N/A	0.00
0	32.5	264.65	N/A	0.00
0.5	31.8	259.47	0.2530	2064.34
0.5	33.3	259.47	0.2709	2110.82
0.5	30.0	259.47	0.2453	2121.60
1	31.4	242.50	0.2708	2091.37
1	31.6	242.50	0.2693	2066.62
1	27.0	242.50	0.2740	2460.93
2	32.2	242.50	0.2275	1713.32
2	30.4	242.50	0.2364	1885.76
2	31.5	242.50	0.2906	2237.16
3	29.6	264.65	0.2840	2539.21
3	29.7	264.65	0.2847	2536.90
3	30.4	264.65	0.3095	2694.38
6	28.8	264.65	0.2582	2372.66
6	29.8	264.65	0.2595	2304.59
6	27.9	264.65	0.2410	2286.04
9	29.7	259.47	0.2424	2117.69
9	31.7	259.47	0.2735	2238.64
9	27.0	259.47	0.2737	2630.26
12	30.4	259.47	0.2658	2268.66
12	30.0	259.47	0.2790	2413.07
12	30.5	259.47	0.2302	1958.36
24	27.6	251.69	0.2817	2568.88
24	29.9	251.69	0.2448	2060.66
24	30.4	251.69	0.2157	1785.84

[a] N/A – Not applicable

Table A-4: Dioxane and HEAA Blood Concentrations (Low Dose Group)

Sacrifice time (hr)	Body Wt. (g)	Actual Dose (mg/kg)	1,4-Dioxane Blood Conc. (μg/g)	1,4-Dioxane Percent Administered Dose (%)[a]	HEAA Blood Conc. (μg/g)
0	29.3	0.00	1.86	N/A[b]	ND[c]
0	31.1	0.00	1.87	N/A	0.49
0	29.4	0.00	0.98	N/A	ND
0.5	25.8	27.68	2.64	0.57	32.29
0.5	29.1	30.29	1.36	0.27	57.16
0.5	30.0	25.53	1.66	0.39	36.61
1	28.2	23.55	1.67	0.43	21.21
1	29.9	22.18	1.28	0.35	ND
1	29.5	21.85	1.83	0.50	ND
2	27.6	22.25	1.66	0.45	3.68
2	30.3	26.29	1.32	0.30	2.01
2	28.6	22.59	1.21	0.32	2.40
3	30.0	20.84	1.41	0.41	ND
3	29.4	22.31	1.92	0.52	0.85
3	26.7	20.23	0.99	0.29	ND
6	31.6	21.57	0.85	0.24	0.43
6	29.8	25.76	1.23	0.29	0.71
6	31.1	22.95	1.24	0.32	ND
9	28.2	29.86	1.34	0.27	ND
9	27.0	27.02	2.41	0.53	ND
9	28.8	22.53	0.93	0.25	ND
12	29.9	19.14	2.18	0.68	ND
12	29.0	24.93	0.91	0.24	ND
12	29.4	23.52	1.03	0.26	ND
24	31.1	27.43	1.32	0.29	ND
24	28.2	25.10	2.00	0.48	ND
24	28.9	26.26	1.73	0.40	ND

[a] Corrected to total blood volume, estimated based on 6% body weight
[b] N/A – Not applicable
[c] ND – Not detected

Table A-5: Dioxane and HEAA Blood Concentrations (Mid Dose Group)

Sacrifice time (hr)	Body Wt. (g)	Actual Dose (mg/kg)	1,4-Dioxane Blood Conc. (µg/g)	1,4-Dioxane Percent Administered Dose (%)[a]	HEAA Blood Conc. (µg/g)
0	ND[b]	0.00	ND	N/A[c]	0.89
0	31.6	0.00	ND	N/A	0.85
0	29.1	0.00	ND	N/A	0.71
0.5	31.2	217.57	96.76	2.67	181.22
0.5	28.4	235.32	118.83	3.03	85.69
0.5	28.9	266.87	99.24	2.23	71.87
1	27.8	272.85	108.36	2.38	160.36
1	26.1	309.57	82.98	1.61	84.15
1	30.2	265.05	82.44	1.87	107.34
2	27.2	235.29	65.95	1.68	97.00
2	31.3	256.41	23.49	0.55	84.24
2	29.8	293.50	41.05	0.84	80.76
3	34.8	222.14	ND	NA	74.72
3	29.9	238.55	9.18	0.23	81.32
3	26.1	297.30	7.92	0.16	59.61
6	29.6	234.56	ND	NA	4.79
6	32.5	230.32	2.49	0.06	3.05
6	29.6	236.14	ND	NA	3.99
9	26.8	268.58	ND	NA	13.11
9	30.8	176.49	ND	NA	2.79
9	28.1	230.08	ND	NA	ND
12	25.1	261.14	ND	NA	ND
12	26.3	N/A	N/A	NA	ND
12	32.1	205.66	ND	NA	ND
24	31.5	217.44	ND	NA	0.12
24	33.1	192.53	ND	NA	ND
24	28.8	282.33	ND	NA	ND

[a] Corrected to total blood volume, estimated based on 6% body weight
[b] ND – Not detected or not determined
[c] N/A – Not applicable

Table A-6: Dioxane and HEAA Blood Concentrations (High Dose Group)

Sacrifice time (hr)	Body Wt. (g)	Actual Dose (mg/kg)	1,4-Dioxane Blood Conc. (µg/g)	1,4-Dioxane Percent Administered Dose (%)[a]	HEAA Blood Conc. (µg/g)
0	29.9	0.00	1.65	N/A[b]	ND[c]
0	28.6	0.00	ND	N/A	1.06
0	32.5	0.00	ND	N/A	ND
0.5	31.8	2064.34	1358.83	3.95	118.95
0.5	33.3	2110.82	1284.10	3.65	149.10
0.5	30.0	2121.60	ND	NA	166.54
1	31.4	2091.37	1850.51	5.31	191.62
1	31.6	2066.62	1577.48	4.58	86.37
1	27.0	2460.93	1758.41	4.29	ND
2	32.2	1713.32	1776.51	6.22	221.82
2	30.4	1885.76	1675.81	5.33	226.42
2	31.5	2237.16	1695.33	4.55	ND
3	29.6	2539.21	1261.77	2.98	191.33
3	29.7	2536.90	1404.10	3.32	201.31
3	30.4	2694.38	1252.26	2.79	214.27
6	28.8	2372.66	1468.71	3.71	200.23
6	29.8	2304.59	1095.27	2.85	231.89
6	27.9	2286.04	1565.07	4.11	243.61
9	29.7	2117.69	852.86	2.42	175.74
9	31.7	2238.64	962.43	2.58	212.47
9	27.0	2630.26	823.42	1.88	ND
12	30.4	2268.66	495.96	1.31	173.56
12	30.0	2413.07	371.40	0.92	164.82
12	30.5	1958.36	283.25	0.87	150.30
24	27.6	2568.88	ND	NA	13.60
24	29.9	2060.66	ND	NA	31.62
24	30.4	1785.84	ND	NA	ND

[a] Corrected to total blood volume, estimated based on 6% body weight
[b] N/A – Not applicable
[c] ND – Not detected